

Amendments to the Claims

1. (currently amended) A method for producing non-2 μ m-family plasmid protein comprising:
 - (a) providing a host cell comprising a 2 μ m-family plasmid, the plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2 μ m-family plasmid protein;
 - (b) culturing the host cell in a culture medium under conditions that allow the co-expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding a non-2 μ m-family plasmid protein; and
 - (c) purifying the thus expressed non-2 μ m-family plasmid protein from the cultured host cell or the culture medium.[;]
2. (original) The method of Claim 1 further comprising the step of formulating the purified non-2 μ m-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.
3. (currently amended) A method Use of producing a non-2 μ m-family plasmid protein comprising:
 - (a) providing a host cell comprising a 2 μ m-family plasmid, the

plasmid comprising a gene encoding protein comprising the sequence of a chaperone protein and a gene encoding a non-2 μ m-family plasmid protein; and

- (b) culturing the host cell in a culture medium under conditions that allow the co-expression of the gene encoding protein comprising the sequence of the chaperone protein and the gene encoding a non-2 μ m-family plasmid protein,

wherein the non-2 μ m-family is an expression vector to increase the production of a fungal (preferably yeast) or vertebrate non-2 μ m-family plasmid protein by providing a gene encoding the non-2 μ m-family plasmid protein and a gene encoding a chaperone protein on the same 2 μ m-family plasmid.

4. (original) A 2 μ m-family plasmid comprising a gene encoding a protein comprising the sequence of a chaperone protein and a gene encoding a non-2 μ m-family plasmid protein, wherein if the plasmid is based on the 2 μ m plasmid then it is a disintegration vector.
5. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the chaperone has a sequence of a fungal chaperone (preferably a yeast chaperone) or a mammalian chaperone (preferably a human chaperone).
6. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the chaperone comprises the sequence of a protein encoded by

any one of *AHA1*, *CCT2*, *CCT3*, *CCT4*, *CCT5*, *CCT6*, *CCT7*, *CCT8*, *CNS1*, *CPR3*, *CPR6*, *EPS1*, *EPO1*, *EUG1*, *FMO1*, *HCH1*, *HSP10*, *HSP12*, *HSP104*, *HSP26*, *HSP30*, *HSP42*, *HSP60*, *HSP78*, *HSP82*, *JEM1*, *MDJ1*, *MDJ2*, *MPD1*, *MPD2*, *PD11*, *PFD1*, *ABC1*, *APJ1*, *ATP11*, *ATP12*, *BTT1*, *CDC37*, *CPR7*, *HSC82*, *KAR2*, *LHS1*, *MGE1*, *MRS11*, *NOB1*, *ECM10*, *SSA1*, *SSA2*, *SSA3*, *SSA4*, *SSC1*, *SSE2*, *SIL1*, *SLS1*, *UBI4*, *ORM1*, *ORM2*, *PER1*, *PTC2*, *PSE1* and *HAC1* or truncated intronless *HAC1*.

7. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the chaperone is protein disulphide isomerase, or comprises the sequence of a protein encoded by *PSE1*, *ORM2* or *SSA1* or a variant or fragment thereof.
8. (currently amended) A method according to ~~any one of~~ Claims ~~1, 2, 5, 6 or 7~~ wherein the host cell also expresses a second recombinant gene encoding a chaperone that is different to the first chaperone encoded by the plasmid.
9. (original) A method according to Claim 8 wherein the second recombinant gene encoding a chaperone is chromosomally integrated.
10. (currently amended) A method, ~~use or plasmid~~ according to ~~any one of~~ Claims ~~1 to 8~~ wherein the plasmid comprises two different genes encoding different chaperones, one of which gene is the second recombinant gene encoding a chaperone as defined by Claim 8.
11. (currently amended) A method, ~~use or plasmid~~ according to ~~any one of~~ Claims

8 to 10 wherein one of the chaperones is protein disulphide isomerase.

12. (currently amended) A method, ~~use or plasmid~~ according to ~~any one of~~ Claims 8 to 11 wherein one of the chaperones is ORM2.
13. (currently amended) A method, ~~use or plasmid~~ according to Claim 8 or 9 wherein the two chaperones are protein disulphide isomerase and ORM2.
14. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the non-2 μ m-family plasmid protein comprises a leader sequence effective to cause secretion in yeast.
15. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the non-2 μ m-family plasmid protein is a eukaryotic protein, or a fragment or variant thereof, preferably a vertebrate or a fungal (such as a yeast) protein.
16. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the non-2 μ m-family plasmid protein is a commercially useful protein.
17. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the non-2 μ m-family plasmid protein comprises a sequence selected from albumin, a monoclonal antibody, an etoposide, a serum protein (such as a blood clotting factor), antistasin, a tick anticoagulant peptide, transferrin, lactoferrin, endostatin, angiostatin, collagens, immunoglobulins, or

Immunoglobulin-based molecules or fragment of either (e.g. a dAb, Fab' fragments, F (ab')₂, scAb, scFv or scFv fragment), a Kunitz domain protein interferons, interleukins, IL10, IL11, IL2, interferon α species and sub-species, interferon β species and sub-species interferon γ species and sub-species, leptin, CNTF, CNTF_{AXIS} (Axokine™), IL1-receptor antagonist, erythropoietin (EPO) and EPO mimics, thrombopoietin (TPO) and TPO mimics, prosaptide, cyanovirin-N, 5-helix, T20 peptide, T1249 peptide, HIV gp41, HIV gpl20, urokinase, prourokinase, tPA, hirudin, platelet derived growth factor, parathyroid hormone, proinsulin, insulin, glucagon, glucagon-like peptides, insulin-like growth factor, calcitonin, growth hormone, transforming growth factor β , tumour necrosis factor, G-CSF, GM-CSF, M-CSF, FGF, coagulation factors in both pre and active forms, including but not limited to plasminogen, fibrinogen, thrombin, pre- thrombin, pro-thrombin, von Willebrand's factor, α_1 -antitrypsin, plasminogen activators, Factor VII, Factor VIII, Factor IX, Factor X and Factor XIII, nerve growth factor, LACI, platelet-derived endothelial cell growth factor (PD-ECGF), glucose oxidase, serum cholinesterase, aprotinin, amyloid precursor protein, inter-alpha trypsin inhibitor, antithrombin III, apo-lipoprotein species, Protein C, Protein S, or a variant or fragment of any of the above.

18. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the non-2 μ m-family plasmid protein comprises the sequence of albumin or a variant or fragment thereof.
19. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceding~~ claim 1 wherein the non-2 μ m-family plasmid protein comprises the sequence of

a transferrin family member, preferably transferrin or lactoferrin, or a variant or fragment thereof.

20. (currently amended) A method, ~~use or plasmid~~ according to ~~any preceeding~~ claim 1 wherein the non-2 μ m-family plasmid protein comprises a fusion protein, such as a fusion protein of albumin or a transferrin family member or a variant or fragment of either, fused directly or indirectly to the sequence of another protein.
21. (currently amended) A host cell comprising a plasmid as defined by ~~any preceeding~~ claim 4.
22. (original) A host cell according to Claim 21 wherein a chaperone encoded by the plasmid is an essential gene.
23. (original) A host cell according to Claim 22 wherein, in the absence of the plasmid, the host cell does not produce the chaperone.
24. (currently amended) A host cell according to ~~any one of Claims 21 to 23 which~~ wherein the host cell is a yeast cell.
25. (original) A host cell according to Claim 24 in which the plasmid is based on pSR1, pSB3 or pSB4 and the yeast cell is *Zygosaccharomyces rouxii*, the plasmid is based on pSB1 or pSB2 and the yeast cell is *Zygosaccharomyces bailli*, the plasmid is based on pSM1 and the yeast cell is *Zygosaccharomyces fermentati*, the plasmid is based on pKD1 and the yeast cell is *Kluyvejomyc*

drosophilarum, the plasmid is based on pPM1 and the yeast cell is *Pichia menabranaefaciens*, or the plasmid is based on the 2 μ m plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*.

26. (original) A host cell according to Claim 25 in which the plasmid is based on the 2 μ m plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*.
27. (currently amended) A method according to Claim 1 wherein the host cell is a host cell as defined by ~~any one of~~ Claims 21 to 26.
28. (currently amended) A method according to Claim 27 wherein the host cell is a host cell as defined by Claim 23, ~~or any other claim dependent thereon~~.
29. (original) A method according to Claim 27 wherein the step (b) involves culturing the host cell in non-selective media, such as a rich media.
30. (original) A method for producing non-2 μ m-family plasmid protein comprising:
 - (a) providing a host cell comprising a first recombinant gene encoding a protein comprising the sequence of a first chaperone protein, a second recombinant gene encoding a protein comprising the sequence of a second chaperone protein and a third recombinant gene encoding a non-2 μ m-family plasmid protein, wherein the first and second chaperones are different;

- (b) culturing the host cell in a culture medium under conditions that allow the expression of the first, second and third genes; and
 - (c) optionally purifying the thus expressed non-2 μ m-family plasmid protein from the cultured host cell or the culture medium; and
 - (d) optionally, lyophilising the thus purified protein.
31. (original) The method of Claim 30 further comprising the step of formulating the purified non-2 μ m-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.
32. (currently amended) A method according to Claims 30 ~~or 31~~ wherein the first and second chaperones comprise the sequence of a protein encoded by any one of *AHA1*, *CCT2*, *CCT3*, *CCT4*, *CCT5*, *CCT6*, *CCT7*, *CCT8*, *CNS1*, *CPR3*, *CPR6*, *EPS1*, *ERO1*, *EUG1*, *FMO1*, *HCH1*, *HSP10*, *HSP12*, *HSP104*, *HSP26*, *HSP30*, *HSP42*, *HSP60*, *HSP78*, *HSP82*, *JEM1*, *MDJ1*, *MDJ2*, *MPD1*, *MPD2*, *PDH1*, *PFD1*, *ABC1*, *APJ1*, *ATP11*, *ATP12*, *BTT1*, *CDC37*, *CPR7*, *HSC82*, *KAR2*, *LHS1*, *MGE1*, *MRS11*, *NOB1*, *ECM10*, *SSA1*, *SSA2*, *SSA3*, *SSA4*, *SSC1*, *SSE2*, *SIL1*, *SLS1*, *UB14*, *ORM1*, *ORM2*, *PER1*, *PTC2*, *PSE1* and *HAC1* or truncated intronless *HAC1*.
33. (currently amended) A method according to ~~any one of~~ Claims 30 ~~to 33~~ wherein the first chaperone is protein disulphide isomerase.

34. (currently amended) A method according to ~~any one of Claims 30 to 34~~ wherein the second chaperone is ORM2.
35. (currently amended) A method according to ~~any one of Claims 30 to 34~~ wherein at least one of the first or second chaperones is encoded by a chromosomally integrated recombinant gene.
36. (currently amended) A method according to ~~any one of Claims 30 to 35~~ wherein at least one of the first or second chaperones is encoded by a gene on a plasmid.
37. (currently amended) A method according to Claim 36 wherein the plasmid is a plasmid as defined by ~~any one of Claim 1 to 26~~ 4.
38. (original) A host cell comprising a first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) and a second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein.
39. (canceled)
40. (currently amended) A host cell according to Claim 38 ~~or use according to Claim 39~~ wherein the transferrin-based protein comprises the sequence of transferrin or any other member of the transferrin family (e.g. lactoferrin), a variant or fragment thereof or a fusion protein comprising transferrin, a variant or fragment thereof.

41. (currently amended) A host cell ~~or use~~ according to ~~any one of~~ Claims 38 ~~to 40~~ wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is provided on a plasmid.
42. (currently amended) A host cell ~~or use~~ according to Claim 41 wherein the plasmid is a 2 μ m-family plasmid.
43. (currently amended) A host cell ~~or use~~ according to ~~any one of~~ Claims 38 ~~to 40~~ wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is chromosomally integrated.
44. (currently amended) A host cell ~~or use~~ according to Claim 43 wherein the first recombinant gene encoding a protein comprising the sequence of protein disulphide isomerase (PDI) is chromosomally integrated at the locus of an endogenously encoded PDI gene, preferably without disrupting the expression of the endogenous PDI gene.
45. (currently amended) A host cell ~~or use~~ according to ~~any one of~~ Claims 38 ~~to 44~~ wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is provided on a plasmid.
46. (currently amended) A host cell ~~or use~~ according to Claim 45 wherein the plasmid is a 2 μ m-family plasmid.
47. (currently amended) A host cell ~~or use~~ according to ~~any one of~~ Claims 38 ~~to 44~~ wherein the second recombinant gene encoding a protein comprising the

sequence of a transferrin-based protein is chromosomally integrated.

48. (currently amended) A host cell ~~or use~~ according to Claim 47 wherein the second recombinant gene encoding a protein comprising the sequence of a transferrin-based protein is chromosomally integrated at the locus of an endogenously encoded PDI gene, preferably without disrupting the expression of the endogenous PDI gene.
49. (original) A method for producing non-2 μ m-family plasmid protein comprising:
- (a) providing a host cell comprising a first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2 μ m-family plasmid protein; and
 - (b) culturing the host cell in a culture medium under conditions that allow the expression of the first and second genes.
50. (original) The method of Claim 49 further comprising the step of formulating the purified non-2 μ m-family plasmid protein with a carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.
51. (currently amended) A method according to Claim 49 ~~or 50~~ wherein the first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof is integrated into the chromosome of the host cell.

52. (currently amended) A method according to Claim 49 ~~or 50~~ wherein the first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof is located on a plasmid.
53. (original) A host cell comprising first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2 μ m-family plasmid protein.
54. (canceled)
55. (original) A plasmid comprising a first recombinant gene encoding a protein comprising the sequence of ORM2 or a variant or fragment thereof and a second recombinant gene encoding a non-2 μ m-family plasmid protein.
56. (original) A plasmid according to Claim 55 which is a 2 μ m-family plasmid.
57. (currently amended) A method, ~~use, host cell or plasmid~~ according to ~~any one of Claims 49 to 56~~ wherein the non-2 μ m-family plasmid protein is as defined in ~~any one of Claims 14 to 20~~.
58. (currently amended) A host cell comprising a plasmid, the plasmid comprising a gene that encodes an essential chaperone wherein, in the absence of the plasmid, the host cell is unable to produce the chaperone, the plasmid further comprising a recombinant gene encoding a non-2 μ m-family plasmid protein, such as a non-2 μ m-family plasmid protein as defined in ~~any one of Claims 14 to 20~~.

59. (original) A host cell according to Claim 58 wherein, in the absence of the plasmid, the host cell is inviable.
60. (currently amended) The host cell of Claim 58 ~~or 59~~ wherein the chaperone is protein disulphide isomerase.
61. (original) A plasmid comprising, as the sole selectable marker, a gene encoding an essential chaperone.
62. (currently amended) The plasmid of Claim 61 further comprising a gene encoding a non-2 μ m-family plasmid protein, such as a non-2 μ m-family plasmid protein as defined in ~~any one of~~ Claims 14 ~~to 20~~.
63. (currently amended) The plasmid of Claim 61 ~~or 62~~ which is a 2 μ m-family plasmid.
64. (currently amended) A method for producing a non-2 μ m-family plasmid protein comprising the steps of :
 - (a) providing a host cell as defined by ~~any one of~~ Claims 58 ~~to 60~~; and
 - (b) culturing the host cell in a culture medium under conditions that allow the expression of the essential chaperone and the non-2 μ m-family plasmid protein.

65. (currently amended) The method of Claim 64 wherein the host cell comprises a plasmid as defined by ~~any one of~~ Claims 61 to 63.
66. (currently amended) The method of Claim 64 ~~or 65~~ wherein step (b) is performed by culturing the host cell in a non-selective medium, such as a rich or complex medium.
67. (canceled)
68. (canceled)
69. (currently amended) A method ~~Use of a polynucleotide comprising the sequence of promoter operably connected to a coding sequence encoding a chaperone~~ for increasing the expression of a non-2 μ m-family plasmid protein in a host cell comprising
- providing a polynucleotide comprising a promoter operably connected to a coding sequence encoding a chaperone; and
- expressing by expression of the polynucleotide sequence within the host cell,
- wherein the promoter is characterised in that it achieves a lower level of expression of the chaperone than would be achieved if the coding sequence were to be operably connected to its naturally occurring promoter.
70. (currently amended) A method for producing a non-2 μ m-family plasmid protein

comprising the steps of :

- (a) providing a host cell comprising a recombinant gene that ~~comprising~~ comprises the sequence of a promoter operably connected to a coding sequence encoding a chaperone, the promoter being characterised in that it achieves a lower level of expression of the chaperone than would be achieved if the coding sequence were to be operably connected to its naturally occurring promoter, and the host cell further comprising a recombinant gene encoding a non-2 μ m-family plasmid protein;
- (b) culturing the host cell ~~in a~~ under conditions that allow the expression of the chaperone and the non-2 μ m-family plasmid protein.

- 71. (original) The method of Claim 1 further comprising the step of lyophilising the thus purified protein.
- 72. (currently amended) The method of Claim 49, ~~64, 68 or 70~~ further comprising the step of purifying the thus expressed non-2 μ m-family plasmid protein from the cultured host cell or the culture medium.
- 73 (original) The method of Claim 72 further comprising the step of lyophilising the thus purified protein.
- 74. (currently amended) The method of Claim 72 ~~or 73~~ further comprising the step of formulating the purified ~~or lyophilized~~ non-2 μ m-family plasmid protein with a carrier or diluent.

75. (original) The method of Claim 74 further comprising the step of presenting the thus formulated protein in a unit dosage form.